Utilizing *Solanum glaucophyllum* Alone or With Phytase to Improve Phosphorus Utilization in Broilers

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ABSTRACT Experiments were conducted to determine if *Solanum glaucophyllum* (SG), a plant containing a glycoside of 1,25-dihydroxyvitamin D, could be used as a feed additive to improve P utilization of broilers. SG leaves (1, 2.5, or 5 g/kg), 1,25-dihydroxyvitamin D (15 μg/kg), or Ca and P (to achieve a 0.92% Ca:0.65% P:0.41% nonphytate P control diet) were added to a 0.56% Ca/0.45% P/0.28% nonphytate P basal diet and fed to broilers from 7 to 28 d of age. Birds fed basal ration alone exhibited reduced weight gain, bone density, and bone mineral content when compared with birds fed the 0.92% Ca:0.65% P diet. Adding 5 g SG leaves or 15 μg of 1,25-dihydroxyvitamin D/kg to the basal diet increased body weight gain, plasma Ca and P, bone ash, and bone density above basal diet levels. Plasma P and weight gain of birds fed 5 g SG or 15 μg of 1,25-dihydroxyvitamin D/kg basal diet were equivalent to those observed in birds fed the 0.92% Ca:0.65% P diet. In experiment 2, the effect of higher doses of SG, as well as the additive effect of SG with 1,200 phytase units/kg diet, were examined in chicks fed a 0.59% Ca and 0.42% P basal diet. Two levels of SG leaves (7.5 g and 10 g), phytase, or both SG (7.5 g) and phytase were added per kilogram of basal diet. Adding SG or phytase to the basal diet increased weight gain, plasma Ca, plasma P, and bone mineral content over that observed in birds fed basal diet alone. Combining SG with phytase provided no significant gains in growth or bone parameters over treatment with phytase alone.

(*Key words*: bone, phosphorus, phytase, *Solanum glaucophyllum*, vitamin D)

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INTRODUCTION

Intensive livestock production is often the target of government regulation as the manure produced during these operations is often perceived as a threat to the environment. Phosphorus in manure that is appropriately applied to the land is adsorbed onto soil particles and utilized by crops. However, excessive application of P in manure can exceed soil-holding capacity and P can leach into waterways. Phosphorus is considered the nutrient that limits growth of fresh-water aquatic plants, especially as it relates to the process of eutrophication (Sharpley et al., 1994). Nearly two-thirds of the P in common feedstuffs is bound to phytate and is largely unavailable to nonruminant animals. This P will be in the manure. The addition of phytase of microbial origin to poultry diets can hydrolyze the phosphoester bonds of phytate-bound P, freeing the P for absorption. Simons et al. (1990) reported that adding phytase to a low P diet increased dietary P availability by 60% and decreased fecal P excretion by 50% in 3-wk-old broiler chicks. Recently, 1,25-dihydroxyvitamin D and synthetic analogs of 1,25-dihydroxyvitamin D were demonstrated to improve P utilization in poultry (Edwards, 1993; Biehl and Baker, 1997; Biehl et al., 1998). These vitamin D metabolites increased P retention, decreased P excretion, and increased bone ash content in the birds. In addition, the incidence and severity of tibial dyschondroplasia was decreased in growing birds by supplementing the diet with 6 μg of 1,25-dihydroxyvitamin D/kg diet (Roberson and Edwards, 1996).

Mitchell and Edwards (1996 a,b) fed broiler chicks diets supplemented with 5 μg of 1,25-dihydroxyvitamin D/kg diet, 600 units of phytase/kg diet, or the combination of 1,25-dihydroxyvitamin D and phytase for up to 35 d. They noted that 1,25-dihydroxyvitamin D or phytase alone could improve P utilization and normalize growth and bone strength when the diet was 0.55% P. They also noted an additive effect when the two were combined, which allowed normal growth and bone development when dietary P was further reduced to 0.45%. Unfortunately, the high cost of 1,25-dihydroxyvitamin D and its synthetic analogs has inhibited investigations to find uses for these compounds in the poultry industry.

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Abbreviation Key: FTU = phytase units; SG = *Solanum glaucophyllum*.

Solanum glaucophyllum (SG) (also known as *Solanum malacoxylon*) is a calcinogenic plant that contains a water-soluble glycoside of 1,25-dihydroxyvitamin D (Wasserman et al., 1976b; Napoli et al., 1977; Boland et al., 1987). This shrub is common in temperate climates of South America and is commonly considered toxic, as grazing animals consuming the leaves can develop severe hypercalcemia and hyperphosphatemia, symptoms identical to those of vitamin D intoxication. We conducted 2 experiments to determine (1) the effect of SG leaf powder, an inexpensive source of 1,25-dihydroxyvitamin D, on P utilization and (2) the additive effect of SG and phytase on P utilization.

MATERIALS AND METHODS

General Procedures

One-day-old Peterson × Arbor Acres (n = 320) broiler chicks were purchased from a commercial hatchery and housed in a brooder. All chicks were fed a diet formulated to be 1% Ca and 0.7% total P that met or exceeded all dietary recommendations as set forth for broiler chicks 0 to 3 wks of age by the National Research Council (NRC, 1994) for the first 7 d after hatching. Upon analyses, these diets were 0.92% Ca, 0.65% P, and 0.41% nonphytate P in experiment 1 and 0.86% Ca, 0.69% P, and 0.46% nonphytate P in experiment 2. These diets were also slightly lower in protein and energy than formulated for. Although they were slightly deficient in protein, energy, and calcium for chicks 0 to 3 wk of age, they exceeded the requirements for chicks 3 to 6 wk of age. On the eighth day, chicks were weighed, and very light and very heavy chicks were eliminated until a more uniform group of 288 chicks remained. These chicks were then randomly distributed in groups of 8 chicks to 1 of 36 floor pens. Each treatment was given to 6 pens randomly distributed throughout the room for the following 21 d. Water and experimental diets were provided ad libitum. The temperature was maintained at 25°C within the barn throughout the experimental period. Feed consumption and weight gain were recorded weekly to assess growth performance. At 28 d of age, all chicks were anesthetized with CO_2:O_2 (50:50). Heparinized blood samples were obtained by cardiac puncture and centrifuged at 3,000 rpm for 20 min. The plasma was collected and stored at –20°C until analyzed. While under anesthesia, chicks were killed by cervical dislocation. These procedures were approved by the Iowa State University and the National Animal Disease Center Institutional Animal Care and Use Committees.

The right tibia was obtained from each chick, and the attached soft tissue was carefully dissected away. Bone density and volume were determined by water displacement as described by Keenan et al. (1992). Briefly, each bone was placed in a beaker of distilled water and then placed into a vacuum chamber overnight so that water would displace air trapped within the bone cavity. The hydrated tibiae were then weighed in water and air (Zhang and Coon, 1997). The bone density was calculated as the bone's weight in air divided by the weight in air minus the weight in water and multiplied by the specific gravity of water (0.997 g/mL) at 25°C. The bone volume (in cm^3) was calculated by dividing each bone's weight in air by its density. Bones from each pen were pooled and dried at 80°C overnight to obtain a dry bone weight. The dried bones were ashed at 600°C for 8 h, and the ash was weighed. Ash was removed from the crucibles using multiple washes with 0.1 *N* hydrochloric acid, and the washes were collected into 50-mL volumetric flasks. Plasma and bone ash Ca concentrations were determined by atomic absorption spectrophotometry (Perkin-Elmer, 1965). Phosphorus concentration was determined colorimetrically (Parekh and Jung, 1970). Plasma 1,25-dihydroxyvitamin D was measured using a commercial RIA kit.[2]

Experiment 1

Dry SG leaves were obtained from Argentina and ground into a powder for incorporation into experimental diets. Six dietary treatments were applied. The negative control treatment consisted of a basal diet containing 0.45% total P (0.28% nonphytate P) and 0.56% Ca (Table 1). This diet met or exceeded all other requirements for nutrients as described by the NRC (1994) for chicks 3 to 6 wk of age but was slightly low in protein and energy for chicks 0 to 3 wk of age. Experimental treatments were 1, 2.5, or 5 g of ground SG leaves added per kilogram of basal diet. Another treatment consisted of adding 15 μg of 1,25-dihydroxyvitamin D/kg of basal diet to compare the activity of the SG leaves with a known amount of 1,25-dihydroxyvitamin D. Sodium phosphate and dicalcium phosphate were added to the basal diet to achieve a 0.92% Ca and 0.65% total P diet (0.41% nonphytate P) that met the requirements for Ca and P for growing broiler chicks 3 to 6 wk of age (NRC, 1994) and served as the positive control diet for the experiment.

Experiment 2

Experiment 2 was designed to determine if SG and phytase might have an additive effect on P utilization. Chicks were fed for the first 7 d posthatch and assigned to treatments as described for experiment 1. The basal diet used was similar to that in experiment 1; however, the P concentration in the basal diet was decreased to 0.42% (0.19% nonphytate P; Table 2) in consideration of the results of experiment 1. We expected added phytase to increase the diet P available to the birds. The results of experiment 1 also suggested that more than 5 g of SG/kg might be required to achieve the same increases in blood and bone parameters achieved by inclusion of synthetic 1,25-dihydroxyvitamin D at 15 μg/kg basal diet. However, the batch of SG leaves used in this experiment was different from the batch used in experiment 1. To

[2]Diasorin, Stillwater, MN.

TABLE 1. Composition of diets fed to broiler chicks during the period from 8 until 28 d of age in experiment 1

Ingredient (% DM)	Basal	Basal + 1 g SG/kg	Basal + 2.5 g SG/kg	Basal + 5 g SG/kg	Basal + 1,25-vitamin D[1]	Control
Corn	51.35	51.23	51.04	50.73	51.35	50.45
Soy meal (44% CP)	39.92	39.91	39.90	39.89	39.92	39.88
Soyoil	5.75	5.75	5.75	5.75	5.75	5.75
Mineral-vitamin premix[2]	0.68	0.68	0.68	0.68	0.68	0.68
Salt	0.22	0.22	0.22	0.22	0.22	0.22
DL-Methionine	0.15	0.15	0.15	0.15	0.15	0.15
Disodium phosphate	0.56	0.56	0.56	0.56	0.56	0
Calcium phosphate	0	0	0	0	0	1.52
Limestone	1.37	1.37	1.37	1.37	1.37	1.36
SG[3]	0	0.10	0.25	0.50	0	0
1,25-$(OH)_2$ vitamin D	0	0	0	0	0.001	0
Composition (90% DM basis)[4]						
Crude protein	22.1	22.1	22.1	22.05	22.1	22.05
ME, kcal/kg	3,091	3,088	3,086	3,074	3,091	3,061
Ether extract	7.65	7.65	7.65	7.65	7.65	7.65
Calcium	0.56	0.56	0.56	0.56	0.56	0.92
Phosphorus	0.45	0.45	0.45	0.45	0.45	0.65
Nonphytate phosphorus[5]	0.28	0.28	0.28	0.28	0.28	0.41

[1]The 1,25-dihydroxyvitamin D was included in the diet at a rate of 15 μg/kg of diet by first incorporating it into the soy oil.

[2]Mineral and vitamin premix provided the following (per kilogram of finished diet): Mn, 70 mg ($MnSO_4 \cdot H_2O$); Zn, 40 mg ($ZnSO_4 \cdot H_2O$); Fe, 37 mg ($FeSO_4 \cdot 7H_2O$); Cu, 6 mg ($CuSO_4 \cdot 5H_2O$); Se, 0.15 mg (Na_2SeO_3); NaCl, 2.6 g (iodized); vitamin A (retinyl acetate), 8,065 IU; cholecalciferol, 1,580 IU; menadione sodium bisulfite, 4 mg; vitamin E 15 IU; vitamin B_{12}, 16 μg; riboflavin, 7.8 mg; pantothenic acid, 12.8 mg; folic acid, 1.62 mg; niacin, 75 mg; biotin, 270 μg; choline chloride, 509 mg.

[3]SG = *Solanum glaucopyllum*.

[4]Crude protein, ME, and ether extract were calculated from analyzed composition of individual feedstuffs (corn, soybean meal) and NRC listed composition of soyoil. Calcium and phosphorus contents listed are the averages obtained upon analysis of 14 basal diet samples and 4 normal diet samples throughout the course of the experiment.

[5]Assumes the corn utilized was 0.08% nonphytate P, and the soybean meal utilized was 0.27% nonphytate P on an as-fed basis.

quantify the relative 1,25-dihydroxyvitamin D activity of the 2 batches of SG, rats were bolus-dosed with ground leaves from the 2 different stocks of SG before the second chick experiment. The plasma concentration of 1,25-dihydroxyvitamin D induced by 1.5 g of SG leaves from the batch to be used in the second experiment was similar to that induced by 1 g of SG leaves from the batch used in experiment 1 (data not shown). Therefore, in experiment 2, we used 7.5 g of SG leaf powder/kg of basal diet (roughly equivalent to the 5-g SG treatment in experiment 1) as one treatment and increased the dose to 10 g of SG leaf powder/kg basal diet as another treatment.

The dietary Ca concentration was 0.59%, similar to experiment 1. Five of the 6 treatments applied across the 36 pens of chicks were basal diet, 7.5 g of SG leaves/kg of basal diet, 10 g of SG leaves/kg of basal diet, 1,200 phytase[3] units (FTU) kg of basal diet, and basal diet with the combination of 1,200 FTU phytase and 7.5 g of SG leaves/kg. Phytase activity added to the diet was based on the label guarantee of the product utilized. The sixth treatment was basal diet supplemented to achieve a diet that was 0.86% Ca, 0.69% total P, and 0.46 nonphytate P as a positive control as in experiment 1 (Table 2).

[3]Natuphos 600, BASF Corporation, Mt. Olive, NJ.

Statistical Analysis

All statistics were conducted on the basis of pen means in which pen was the experimental unit. Treatment means were analyzed by ANOVA, and significant differences among treatment means were assessed using the Fisher's least significant difference multiple pairwise comparison procedure with a 5% level of probability.

RESULTS

Experiment 1

Weight gain, plasma P, bone mineral content, and bone density were significantly reduced in birds fed basal diet when compared with birds fed the control diet (Tables 3 and 4). Adding 15 μg of 1,25-dihydroxyvitamin D or 5 g of SG to the basal ration increased weight gain and plasma P to concentrations similar to those observed in birds fed the control diet ($P < 0.01$) (Table 3). No significant differences in feed efficiency or plasma Ca concentration were observed across all treatments.

Bone density was increased by all SG treatments when compared with the basal diet group ($P < 0.01$). Addition of 5 g of SG/kg basal diet improved dry bone weight (data not shown) and tibia ash weight (Table 4) to levels observed in birds fed the control diet but failed to com-

TABLE 2. Composition of diets fed to broiler chicks during the period from 8 until 28 d of age in experiment 2

Ingredient (% DM)	Basal	Basal + 7.5 g SG/kg	Basal + 10 g SG/kg	Basal + phytase[1]	Basal + phytase + SG	Control
Corn	50.48	49.75	49.42	49.78	49.48	49.25
Soy meal (44% CP)	41.39	41.37	41.33	41.79	41.34	41.34
Soyoil	5.76	5.75	5.75	5.81	5.75	5.75
Mineral-vitamin premix[2]	0.68	0.68	0.68	0.68	0.68	0.68
Salt	0.22	0.22	0.22	0.22	0.22	0.22
DL-Methionine	0.15	0.15	0.15	0.15	0.15	0.15
Disodium phosphate	0.21	0.21	0.21	0.21	0.21	0
Calcium phosphate	0	0	0	0	0	0.51
Limestone	1.11	1.11	1.11	1.12	1.11	1.11
SG[3]	0	0.75	1.0	0	0.75	0
Phytase	0	0	0	0.23	0.22	0
Composition (90% DM basis)[4]						
Crude protein	22.7	22.7	22.6	22.6	22.6	22.6
ME, kcal/kg	3,096	3,067	3,056	3,082	3,054	3,052
Ether extract	7.65	7.65	7.65	7.65	7.65	7.65
Calcium	0.59	0.59	0.59	0.59	0.59	0.86
Phosphorus	0.42	0.42	0.42	0.42	0.42	0.69
Nonphytate phosphorus[5]	0.19	0.19	0.19	0.19	0.19	0.46

[1]Phytase concentrate was included in the diet to provide 1,200 phytase units/kg of diet.

[2]Mineral and vitamin premix provided the following (per kilogram of finished diet): Mn, 70 mg ($MnSO_4 \cdot H_2O$); Zn, 40 mg ($ZnSO_4 \cdot H_2O$); Fe, 37 mg ($FeSO_4 \cdot 7H_2O$); Cu, 6 mg ($CuSO_4 \cdot 5H_2O$); Se, 0.15 mg (Na_2SeO_3); NaCl, 2.6 g (iodized); vitamin A (retinyl acetate), 8,065 IU; cholecalciferol, 1,580 IU; menadione sodium bisulfite, 4 mg; vitamin E 15 IU; vitamin B_{12}, 16 μg; riboflavin, 7.8 mg; pantothenic acid, 12.8 mg; folic acid, 1.62 mg; niacin, 75 mg; biotin, 270 μg; choline chloride, 509 mg.

[3]SG = *Solanum glaucopyllum*.

[4]Crude protein, ME, and ether extract were calculated from analyzed composition of major feedstuffs (corn, soybean meal) and NRC listed composition of soyoil. Calcium and phosphorus contents listed are the averages obtained upon analysis of 5 basal diet samples and 2 normal diet samples throughout the course of the experiment.

[5]Assumes that corn utilized was 0.08% nonphytate P, and the soybean meal utilized was 0.27% nonphytate P on an as-fed basis.

pletely normalize bone Ca and P contents. Treatment with 15 μg of 1,25-dihydroxyvitamin D/kg of basal diet normalized bone density, bone ash content, bone Ca content, and bone P content to those levels observed in birds fed the control diet.

Experiment 2

Though the birds fed basal diet had significantly lower plasma P concentration than did birds fed all the other dietary treatments, weight gain in these birds was similar to that of birds fed the control diet (Table 5). Addition of SG or phytase to the basal diet increased plasma P concentrations to concentrations similar to those observed in birds fed the control diet. Addition of phytase or the combination of SG and phytase to the basal diet increased weight gain above that observed in the birds fed the control diet. Weight gain of birds fed basal diet with phytase was significantly greater than weight gain of birds fed the basal diet supplemented with SG only ($P < 0.001$). There were no significant differences in feed efficiency among dietary treatment groups. Plasma 1,25-

TABLE 3. Experiment 1. Effect of *Solanum glaucophyllum* (SG) and 1,25-dihydroxycholecalciferol on growth performance and plasma parameters in broilers when added to a basal diet that was 0.56% calcium, 0.45% phosphorus, and adequate vitamin D_3[1]

			Plasma[3]	
Diet[2]	Weight gain (g)	Gain/feed (g/kg)	Calcium (mg/dL)	Phosphorus (mg/dL)
Basal	1,213 ± 23[b]	663 ± 83	10.2 ± 0.2	7.1 ± 0.1[c]
Basal + 1 g SG/kg	1,260 ± 18[b]	677 ± 33	10.4 ± 0.2	7.3 ± 0.2[c]
Basal + 2.5 g SG/kg	1,213 ± 18[b]	696 ± 19	10.4 ± 0.2	7.7 ± 0.2[b]
Basal + 5 g SG/kg)	1,286 ± 16[a]	679 ± 27	10.3 ± 0.2	8.1 ± 0.2[ab]
Basal + 15 μg 1,25-$(OH)_2D_3$/kg	1,298 ± 16[a]	714 ± 25	10.3 ± 0.2	8.3 ± 0.2[a]
Control	1,278 ± 14[a]	668 ± 100	10.3 ± 0.1	8.0 ± 0.1[a]

[a–c]Means within a column lacking a common superscript are significantly different ($P < 0.05$).

[1]Means ± SEM of weight gain and gain/feed of 6 pens (8 chicks/pen) during the period from 8 to 28 d posthatch; average initial weight was 156 g/chick.

[2]1,25-$(OH)_2D_3$ = 1,25 dihyroxyvitamin D.

[3]Plasma samples obtained from chicks at 28 d of age.

TABLE 4. Experiment 1. Effect of *Solanum glaucophyllum* (SG) and 1,25-dihydroxycholecalciferol on bone mineralization in broilers when added to a basal diet that was 0.56% calcium, 0.45% total phosphorus, and adequate in vitamin D_3[1]

Diet[2]	Tibia			Mineral content	
	Ash		Density	Calcium	Phosphorus
	(g)	(%)	(g/cm^3)	(mmol/cm^3)	
Basal	1.61 ± 0.03^{d}	38.5 ± 0.3^{bc}	1.139 ± 0.003^{c}	1.35 ± 0.17^{c}	0.84 ± 0.15^{bc}
Basal + 1 g SG/kg	1.69 ± 0.02^{d}	37.7 ± 0.2^{c}	1.148 ± 0.001^{b}	1.34 ± 0.20^{c}	0.82 ± 0.18^{c}
Basal + 2.5g SG/kg	1.66 ± 0.03^{d}	38.9 ± 0.6^{bc}	1.148 ± 0.002^{b}	1.37 ± 0.21^{bc}	0.83 ± 0.13^{bc}
Basal + 5g SG/kg	1.75 ± 0.03^{c}	38.4 ± 0.5^{bc}	1.149 ± 0.002^{b}	1.41 ± 0.13^{b}	0.86 ± 0.12^{b}
Basal + 15 μg 1,25-$(OH)_2D_3$/kg	1.86 ± 0.03^{b}	39.7 ± 0.5^{b}	1.163 ± 0.002^{a}	1.53 ± 0.22^{a}	0.93 ± 0.11^{a}
Control	1.96 ± 0.04^{a}	41.9 ± 0.2^{a}	1.162 ± 0.003^{a}	1.55 ± 0.12^{a}	0.94 ± 0.16^{a}

$^{a–d}$Means within a column lacking a common superscript are significantly different ($P < 0.05$).

[1]Means ± SEM of 6 pens (8 chicks/pen) of bone parameters at 28 d posthatch; average initial weight was 156 g/chick.

[2]1,25-$(OH)_2D_3$ = 1,25 dihyroxyvitamin D.

dihydroxyvitamin D concentration was increased in birds fed SG when compared with birds fed control diet, but the mean plasma 1,25-dihydroxyvitamin D concentration was not significantly different from that observed in birds fed basal diet only. No additive effect of SG and phytase was observed in either growth performance or plasma P. However, the combination of SG and phytase did improve plasma Ca over treatment with phytase alone.

Addition of SG, phytase, or both to the basal diet significantly increased tibia ash weight, density, and mineral content above that observed in birds fed basal diet only ($P < 0.005$, Table 6). The effects of 10 g of SG/kg of basal diet on bone were not significantly different from the effects of 7.5 g of SG/kg of basal diet. Phytase induced greater effects on bone ash than either of the SG treatments alone ($P < 0.005$). Phytase alone or SG alone had similar effects on bone density and mineral content. The effect of the combination of SG and phytase on bone was not statistically different from the effect of phytase alone, although there was a tendency for bone density, ash, and bone calcium content to be further improved by the combined SG and phytase treatment. Combining phytase and SG resulted in greater bone ash and bone calcium content than did adding SG alone to the diet ($P < 0.05$). None of the treatments completely normalized bone parameters when compared with the bone parameters observed in the control diet birds.

DISCUSSION

It has previously been demonstrated that SG is a source of 1,25-dihydroxyvitamin D activity. Extracts of SG leaves restored intestinal Ca-binding protein synthesis and, hence, increased Ca absorption in vitamin D-deficient chicks and rats in the same manner as 1,25-dihydroxyvitamin D (Wasserman et al., 1976a; Schneider and Schedl, 1977).

We reduced the dietary calcium content of our basal diet from the NRC-recommended concentration of 1 to 0.6%, which appears to have prevented any SG-induced hypercalcemia in the chicks in our study. In preliminary studies we had observed that hypercalcemia associated with SG ingestion in birds fed 1% Ca diets caused a severe reduction in feed intake (data not shown). Birds receiving SG or 1,25-dihydroxyvitamin D treatments that were fed these low Ca diets were able to maintain normal plasma Ca concentration while still improving bone mineral content.

TABLE 5. Experiment 2. Effect of *Solanum glaucophyllum* (SG) and phytase on growth performance and plasma parameters in broilers when added to a vitamin D_3-adequate basal diet containing 0.59% calcium and 0.42% total phosphorus[1]

Diet	Weight gain	Gain:feed	Plasma[2]		
			Calcium	Phosphorus	1,25-$(OH)_2D_3$
	(g)	(g/kg)	(mg/dL)		(pg/mL)
Basal	986 ± 16^{b}	688 ± 13	9.2 ± 0.2^{ab}	4.6 ± 0.3^{b}	157 ± 7^{a}
Basal + 7.5 g SG/kg	1,045 ± 31^{b}	697 ± 16	9.5 ± 0.2^{a}	6.9 ± 0.3^{a}	176 ± 14^{a}
Basal + 10 g SG/kg	1,046 ± 23^{b}	702 ± 15	9.2 ± 0.1^{ab}	7.0 ± 0.3^{a}	170 ± 8^{a}
Basal + 1,200 FTU[3] phytase/kg	1,136 ± 11^{a}	727 ± 7	8.9 ± 0.1^{b}	7.1 ± 0.2^{a}	128 ± 7^{b}
Basal + 7.5 g SG/kg + 1,200 FTU phytase/kg	1,124 ± 16^{a}	727 ± 6	9.4 ± 0.2^{a}	7.5 ± 0.2^{a}	143 ± 11^{ab}
Control	1,033 ± 20^{b}	685 ± 24	9.0 ± 0.1^{ab}	7.5 ± 0.3^{a}	81 ± 4^{c}

$^{a–c}$Means within a column lacking a common superscript are significantly different ($P < 0.05$).

[1]Means ± SEM of weight gain and gain/feed of 6 pens (8 chicks/pen) during the period from 8 to 28 d posthatch; average initial weight was 133 g/chick.

[2]Plasma samples obtained from chicks at 28 d of age.

[3]Phytase units.

TABLE 6. Experiment 2. Effect of *Solanum glaucophyllum* (SG) and phytase on bone mineralization variables in broilers when added to a vitamin D_3-adequate diet containing 0.59% calcium and 0.42% total phosphorus[1]

Diet	Tibia			Mineral content	
	Ash		Density	Calcium	Phosphorus
	(g)	(%)	(gm/cm^3)	($mmol/cm^3$)	
Basal	1.02 ± 0.03[d]	36.8 ± 0.4[d]	1.117 ± 0.007[c]	1.01 ± 0.02[d]	0.68 ± 0.02[c]
Basal + 7.5 g SG/kg	1.19 ± 0.03[c]	37.9 ± 0.2[cd]	1.132 ± 0.003[b]	1.18 ± 0.02[c]	0.78 ± 0.01[b]
Basal + 10 g SG/kg	1.25 ± 0.05[c]	38.7 ± 0.5[c]	1.128 ± 0.002[bc]	1.19 ± 0.02[c]	0.80 ± 0.01[b]
Basal + 1,200 FTU[2] phytase/kg	1.35 ± 0.02[b]	40.8 ± 0.8[b]	1.136 ± 0.002[b]	1.24 ± 0.01[bc]	0.81 ± 0.02[b]
Basal + 7.5 g SG/kg + 1,200 FTU phytase/kg	1.38 ± 0.02[b]	40.4 ± 0.3[b]	1.137 ± 0.001[b]	1.29 ± 0.02[b]	0.80 ± 0.03[b]
Control	1.49 ± 0.03[a]	42.8 ± 0.03[a]	1.158 ± 0.006[a]	1.39 ± 0.04[a]	0.92 ± 0.02[a]

[a–d]Means within a column lacking a common superscript are significantly different ($P < 0.05$).

[1]Means ± SEM of 6 pens (8 chicks/pen) of bone parameters at 28 d posthatch; average initial weight was 133 g/chick.

[2]Phytase units.

Administration of 1,25-dihydroxyvitamin D can also enhance P absorption. The enhanced P absorption is associated with increased transcription of DNA to RNA and increased protein synthesis on the lumenal side of the intestinal epithelium (Basudde and Humphreys, 1975; Haussler et al., 1977; Peterlik and Wasserman, 1978). However, a specific transport mechanism for phosphate absorption remains unidentified. In experiment 1, we demonstrated that 5 g of SG leaves/kg of diet could normalize plasma P in broiler chicks fed a low Ca, low P, corn-soybean-based diet nearly as well as 15 μg of 1,25-dihydroxyvitamin D/kg of basal diet. In parathyroidectomized rats (Campos et al., 1973) and in rabbits (Basudde and Humphreys, 1975), SG is capable of enhancing intestinal P absorption and increasing blood P concentration, in agreement with our results. Birds fed basal diet alone in experiment 2 had elevated plasma concentrations of 1,25-dihydroxyvitamin D when compared with birds on the control diet. Low blood phosphorus alone can stimulate renal production of 1,25-dihydroxyvitamin D as an attempt to maintain P homeostasis (Gray and Garthwaite, 1985). The addition of SG to the diet elevated plasma 1,25-dihydroxyvitamin D concentrations in the birds, and these concentrations were maintained even as plasma P concentrations returned toward normal.

A stimulatory effect of SG on P absorption has not been observed in all studies. Ross et al. (1971), in a toxicity study, reported that the vitamin D activity of SG could cause hypercalcemia in chicks fed 5 g of SG/kg of diet for 3 wk. However, they did not observe any increase in blood P concentration above normal concentrations as a result of SG treatment. Their observations are likely due to the relatively high Ca and P content of the diet used in their studies (approximately 1.27% Ca and 1.0% P). Vitamin D toxicity is usually accompanied by hyperphosphatemia and hypercalcemia, and SG toxicity in grazing ruminants is characterized by hypercalcemia and hyperphosphatemia (Boland, 1988). Canas et al. (1977) observed that methanol extracts of SG leaves were able to enhance Ca absorption in a rachitic chick model but had no ability to enhance intestinal P absorption or restore normal blood P concentrations, although bone ash was restored to normal. The doses of SG used in those studies were between 250 and 1,000 mg of SG leaves per day, similar to the amount of SG ingested by the birds in this study (5 g of SG/kg of diet supplied about 500 mg of SG/bird per day). The authors suggested that SG might not be capable of activating intestinal P absorption mechanisms as effectively as does 1,25-dihydroxyvitamin D. It is difficult to explain why the study of Canas et al. (1977) failed to observe an increase in P absorption, whereas our study suggested a stimulatory effect of SG on P absorption. Perhaps blood P did not increase in their rachitic chicks treated with SG because any absorbed P was being utilized to form new bone.

Norrdin et al. (1979) reported the acute effect of SG in growing rats was stimulation of trabecular bone formation characterized by an increase in the bone apposition rate on trabecular surfaces. In experiment 2, tibia ash content and bone density were increased when SG was added to the Ca- and P-deficient diet. However, bone ash can be a misleading measure of bone response because total bone growth can also increase without altering the percentage of bone ash. Bone composition per unit of volume ($mmole/cm^3$) provides a better indication of the magnitude of mineral changes in bone tissue (Keenan et al., 1992). Compared with the basal treatment, the addition of at least 5 g of SG/kg of diet significantly increased bone Ca and bone P concentrations (Tables 4 and 6), indicating improvement of bone mineralization.

In experiment 1, addition of 15 μg of 1,25-dihydroxyvitamin D/kg to the 0.56% Ca:0.45% P diet (0.28% nonphytate P) restored bone density and bone mineral content to that of birds fed the diet meeting NRC requirements for P. Edwards (1993) reported that 5 μg of 1,25-dihydroxyvitamin D in a low P diet greatly increased bone ash and Ca and P retention and decreased the incidence of rickets when fed over a 9-d experimental period. With the same amount of 1,25-dihydroxyvitamin D, the incidence and severity of tibial dyschondroplasia was also decreased (Mitchell and Edwards, 1996a,b). Biehl et al. (1995) demonstrated 10 μg of 1,25-dihydroxyvitamin D increased bone ash and body weight in 2-wk-old chicks fed 0.63% Ca:0.43% total P (0.1% nonphytate P).

Although plasma P was normalized in experiment 1 by treatment with 15 μg of 1,25-dihydroxyvitamin D or

5 g of SG leaves/kg of basal diet, bone density and bone mineral content remained below normal in the birds treated with 5 g of SG/kg of basal diet. Therefore, in experiment 2, we increased the SG dosage from 7.5 to 10 g/kg of basal diet, after adjusting for the difference in activity of the 2 batches of SG leaves. The effects of a higher dose of SG (10 g/kg of basal diet) on bone and blood were not significantly different from the effects of 7.5 g of SG/kg of basal diet (Tables 5 and 6). Perhaps the further reduction in basal dietary P from 0.45 to 0.42% in experiment 2 (reducing nonphytate P from 0.28 to 0.19%) prevented any further effect of a source of 1,25-dihydroxyvitamin D activity from becoming apparent. Another possibility is a limited ability of the chick digestive tract to break the glycoside linkage with 1,25-dihydroxyvitamin D. We do not have a good gauge of the efficiency with which the chick frees 1,25-dihydroxyvitamin D from SG leaves. However, it is clear that SG leaves can serve as a source of 1,25-dihydroxyvitamin D in poultry diets, and SG can be utilized to improve Ca and P utilization.

As reported in numerous studies (Nelson et al., 1971; Qian et al., 1977), adding phytase increased bone mineral content, blood P, and growth performance in chicks fed a low P diet. As shown in Tables 5 and 6, adding 1,200 FTU of phytase/kg of basal diet increased weight gain, plasma P, tibiae ash, and bone mineral content to a greater content than the SG treatments, suggesting phytase is superior to SG in enhancement of dietary P utilization.

An additive effect of vitamin D metabolites with phytase has been reported. Biehl et al. (1995) observed that adding 1,200 FTU phytase and 10 μg of 1,25-dihydroxyvitamin D/kg to a 0.63% Ca:0.43% P diet (0.1% non-phytate P) increased bone ash more than either treatment alone. Mitchell and Edwards (1996a,b) reported that the addition of 5 μg of 1,25-dihydroxyvitamin D and 600 FTU of phytase/kg to a 0.45% P basal ration increased body weight and bone ash and decreased incidence of rickets more than either treatment alone.

With the exception of some further improvement in plasma Ca concentration, our study failed to demonstrate an additive effect of 1,25-dihydroxyvitamin D and phytase in a 0.59% Ca:0.19% nonphytate P diet. Perhaps because inclusion of 1,200 FTU of phytase/kg diet normalized blood Ca and P, it was impossible for additional 1,25-dihydroxyvitamin D to have any effect on these parameters. Phytase alone did not completely normalize the bone parameters. Bone Ca content was slightly improved ($P < 0.10$) by the combination of SG and phytase over bone Ca content when phytase alone was added, but overall combining SG with phytase did not further improve bone parameters.

We chose the 1,200 FTU dose of phytase to duplicate the work of Biehl et al. (1995), utilizing SG as a source of 1,25-dihydroxyvitamin D instead of adding synthetic 1,25-dihydroxyvitamin D as they did. At 1,200 FTU of phytase/kg of diet, our rate of supplementation was higher than the 300 FTU of phytase/kg of diet used by the poultry industry (McKnight, 1996). A lower dose of phytase might have allowed an additive effect of 1,25-dihydroxyvitamin D to manifest itself. Phytase is clearly an effective means of improving P utilization, and its use should be encouraged. Even if an additive effect of 1,25-dihydroxyvitamin D (or SG) and phytase does not exist, there still may be some incentive to use both. As the studies by Mitchell and Edwards (1996a) and Rennie and Whitehead (1996) demonstrate, another benefit of adding a source of 1,25-dihydroxyvitamin D activity to the diet may be a reduction in the incidence of tibial dyschondroplasia. Biehl et al. (1995) also suggested that 1,25-dihydroxyvitamin D may improve utilization of trace minerals.

Adding phytase, 1,25-dihydroxyvitamin D, or SG (a source of 1,25-dihydroxyvitamin D) to the diet of broilers can improve the efficiency of absorption and utilization of P, thus allowing producers to formulate diets lower in P and reduce P excreted in poultry manure. Addition of 1,25-dihydroxyvitamin D or SG also improves Ca utilization, allowing formulation of diets lower in Ca, which may allow slight increases in energy or protein concentration of the ration. Approval issues aside, addition of 1,25-dihydroxyvitamin D to poultry rations is currently too expensive for practical use. Leaves or extracts of SG leaves may prove an inexpensive means of adding 1,25-dihydroxyvitamin D activity to poultry diets to reduce mineral supplement costs to the poultry industry while reducing potential negative impacts of poultry manure on the environment.

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